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The cytotoxicity and genotoxicity of soluble and particulate cobalt in human lung fibroblast cells

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ABSTRACT

Cobalt exposure is increasing as cobalt demand rises worldwide due to its use in enhancing rechargeable battery efficiency, super-alloys, and magnetic products. Cobalt is considered a possible human carcinogen with the lung being a primary target. However, few studies have considered cobalt-induced toxicity in human lung cells. Therefore, in this study, we sought to determine the cytotoxicity and genotoxicity of particulate and soluble cobalt in human lung cells. Cobalt oxide and cobalt chloride were used as representative particulate and soluble cobalt compounds, respectively. Exposure to both particulate and soluble cobalt induced a concentration-dependent increase in cytotoxicity, genotoxicity, and intracellular cobalt ion levels. Based on intracellular cobalt ion levels, we found that soluble cobalt was more cytotoxic than particulate cobalt while particulate and soluble cobalt induced similar levels of genotoxicity. However, soluble cobalt induced cell cycle arrest indicated by the lack of metaphases at much lower intracellular cobalt concentrations compared to cobalt oxide. Accordingly, we investigated the role of particle internalization in cobalt oxide-induced toxicity and found that particle-cell contact was necessary to induce cytotoxicity and genotoxicity after cobalt exposure. These data indicate that cobalt compounds are cytotoxic and genotoxic to human lung fibroblasts, and solubility plays a key role in cobalt-induced lung toxicity.

Introduction

The demand for cobalt is rising worldwide. Cobalt is increasingly being used in the production of rechargeable batteries, super-alloys and magnetic products due to its enhanced electrode conductivity, anti-corrosive, high melting point and magnetic properties, (CDI, 2013). Based on World Bureau of Metal Statistics, the demand for cobalt has increased by 15% worldwide from 2010 to 2011 alone (CDI, 2013). As cobalt is increasingly used in manufacturing, the potential for exposure of both industrial workers and the general population is also rising.

The International Agency for Research on Cancer (IARC) has classified cobalt as a Group 2B possible human carcinogen (IARC, 2006). Epidemiological studies on the carcinogenicity of cobalt are limited and inconclusive, partially due to co-exposure with established carcinogens, such as nickel and chromium (IARC, 2006). However, human studies do suggest a correlation between cobalt exposure and lung diseases, including lung cancer, asthma and alveotitis (Mur et al., 1987; Sauni et al., 2010; Van Cutsem et al., 1987). Additionally, animal studies conducted by the National Toxicology Program (1998) demonstrated an increased incidence of alveolar/bronchiolar neoplasms in murine models as a result of cobalt inhalation exposure (ATSDR, 2004; IARC, 2006; NTP, 1998).

The potential mechanisms of cobalt-induced lung carcinogenesis remain unknown. Studies indicate that cobalt is genotoxic to human and rodent cells, inducing chromosome aberrations, single and double strand breaks, sister chromatid exchanges and micronuclei (reviewed in ATSDR, 2004; Beyersmann and Hartwig, 2008; Lison et al., 2001). However, despite the fact that the lung is the major target organ, little is known about the genotoxic effects of cobalt in human lung cells. Only one study has investigated the genotoxicity of cobalt in human lung cells and found that exposure to soluble cobalt ions induces DNA double strand breaks in the cancer-derived H460 human lung epithelial cell line (Pastel et al., 2012). Thus far, no studies have investigated cobalt-induced genotoxicity in a non-cancerous human lung cell model.

Both soluble and particulate cobalt compounds are used in industry, but the role of solubility in cobalt-induced genotoxicity remains unknown. Studies with other metals, such as nickel and chromium, indicate that solubility can play an important role in the potency of metal-induced genotoxicity and carcinogenicity. To date, no studies have compared the potency of soluble and particulate cobalt compounds

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in human lung cells. Accordingly, the objective of this study was to determine the cytotoxicity and genotoxicity of soluble and particulate cobalt in human lung fibroblast cells and to investigate the role of solubility in cobalt-induced toxicity.

Materials and methods

Chemicals and reagents

A 50:50 mixture of Dulbecco's minimal essential medium and Ham's F-12 (DMEM/F-12) was purchased from Mediatech Inc. (Herndon, VA). Sodium pyruvate, penicillin/streptomycin, Gluta-Gro, trypsin/EDTA, phosphate buffered saline (PBS), propidium iodide (PI), and Gurr's buffer were purchased from Life Technologies Corp (Carlsbad, CA). Acetic acid, crystal violet, and methanol were purchased from J.T. Baker (Phillipsburg, NJ). Cosmic calf serum (CCS) was purchased from Hyclone (Logan, UT). Cobalt (II) chloride hexahydrate, cobalt (II) oxide, potassium chloride (KCl), and demecolchicine were purchased from Sigma/Aldrich (St. Louis, MO). Giesma stain was purchased from Biomedical Specialties Inc. (Santa Monica, CA). Sodium dodecyl sulfate (SDS) was purchased from American Bioanalytical (Natick, MA). Tissue culture dishes, flasks and plastic ware were purchased from Becton, Dickinson and Company (Franklin Lakes, NJ).

Cells and cell culture

A human lung fibroblast cell line, WTHBF-6, was used as the cell model for this study. WTHBF-6 cells are an hTERT-immortalized clonal cell line derived from primary human bronchial fibroblasts. They exhibit a stable, normal diploid karyotype and have similar clastogenic and cytotoxic responses to metals as their parent cells (Wise et al., 2004). WTHBF-6 cells were cultured as sub-confluent monolayers in DMEM/F-12 supplemented with 15% cosmic calf serum, 100 u/ml penicillin/100 ug/ml streptomycin, 2 mM gluta-GRO, and 0.1 mM sodium pyruvate and incubated in 5% CO_2 at 37 °C. Media were replaced with fresh, warm media every two days. Cells were subcultured every three to four days using 0.25% trypsin/1 mM EDTA solution.

Cobalt preparation

Cobalt oxide (CAS #1307-96-6) was used as a representative particulate cobalt compound and was administered as a suspension in water, as previously described (Wise et al., 2002). Briefly, cobalt oxide was suspended in cold sterile-filtered water and spun overnight with a magnetic stir bar. Dilutions were made from the stock using a vortex mixer and appropriate volumes were dispensed into cell cultures. Cobalt chloride hexahydrate (CAS #7791-13-1) was used as a representative soluble cobalt compound. Stock cobalt chloride solutions were filtered through a 0.2 μM filter, and then appropriate dilutions were made with sterile-filtered water and administered to the cells. It is important to choose treatment doses relevant to actual exposure concentrations that humans might encounter. The Occupational Safety and Health Administration's permissible exposure limit (OSHA PEL) for cobalt is 100 μg/m^3. A human exposed to 100 μg/m^3 for eight hours with an average daily air consumption of 20 m^3 (6.67 m^3 in eight hours) could potentially be exposed to up to approximately 667 μg of cobalt each day occupationally. Treatment dilution concentrations were chosen to be less than the OSHA PEL for cobalt, which is presumed to be the amount of cobalt a human would occupationally be exposed to in a 24 hour period.

Particle size distribution

Cobalt oxide particle size distributions were determined using a Malvern-2000S (Mastersizer) on the basis of number, volume and scattering intensity. Particle sizes ranged from 0.27 μm to 3.56 μm, with an average particle size of 1 μm.

Cytotoxicity assay

Cytotoxicity was determined by a clonogenic assay as previously described (Wise et al., 2002). Briefly, cells were seeded at a density of 9000 cells/cm^2 in a tissue-culture dish, and allowed to grow for 48 h. The cultures were then treated for 24 h with cobalt compounds. Cells were then removed from the dish and reseeded at colony forming density (1000 cells per dish). Colonies were allowed to grow for 14 days; fixed with 100% methanol; stained with crystal violet; and the colonies with at least 50 cells were counted. There were four dishes per treatment group and each experiment was repeated at least three times. Results are expressed as relative survival reflecting the number of colonies within a treatment group divided by the negative control.

Clastogenicity assay

Cobalt-induced clastogenicity was measured using the chromosome aberration assay, as previously described (Wise et al., 2002). Briefly, cells were seeded at a density of 9000 cells/cm^2 in a tissue-culture dish, and allowed to grow for 48 h. The cultures were then treated with varying concentrations of cobalt chloride or cobalt oxide for 24 h exposure periods and harvested for metaphases. One hundred metaphases per data point were analyzed in each experiment and each experiment was repeated at least three times. Metaphases were analyzed for chromatid breaks, isochromatid breaks, chromatid exchanges, dicentrics, double minutes, acentric fragments, fragmented chromosomes and centromere spreading.

Intracellular and extracellular cobalt ion measurements

Intracellular cobalt ion levels were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) as previously described (Holmes et al., 2005). Briefly, cells were seeded at a density of 9000 cells/cm^2 in a tissue-culture dish, and allowed to grow for 48 h. The cultures were then treated with varying concentrations of cobalt chloride for 24 h or cobalt oxide for 0 h or 24 h exposure periods. After treatment, cells were harvested and placed in hypotonic solution followed by 2% SDS to degrade the cellular membrane. This solution was sheered through an 18 gauge needle and filtered through a 0.2 μm filter. Samples were diluted in 2% nitric acid and cobalt ion concentrations were measured by ICP-OES as previously described (Holmes et al., 2005).

The 0 h treatment for particulate cobalt was performed to account for the possibility that cobalt particles may have passed through the 0.2 μm filter. This potential confounding factor was accounted for by subtracting the 0 h cobalt ion levels from the 24 h cobalt ion levels. The corrected intracellular concentrations were converted from ug/L to μM by dividing by the volume of the sample, the atomic weight of the chemical, the number of cells in the sample and the average cell volume (determined to be 1.125 pl by a Beckman Coulter Multisizer 3).

Statistics

Student's t-tests were conducted to determine statistical significance between data points. Statistical significance was determined to be a p value less than 0.05.

Results

Soluble cobalt is more cytotoxic to human lung cells than particulate cobalt

Exposure to particulate or soluble cobalt induced a concentration-dependent increase in cytotoxicity in human lung fibroblasts (Fig. 1).

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